THE TERMINATIONS OF THE NERVES IN THE TESTICLE.

By H. G. BEYER, M.D., M.R.C.S.,

PASSED ASSISTANT SURGEON U. S. NAVY.

THE nerves of the testicle and their terminations not having received their deserved attention from histologists has prompted me to make a series of experiments which, it is hoped, may throw more light on this interesting subject.

This want of success is probably due mainly to two causes: first, the imperfectness and uncertainty of the existing methods employed in tracing out the finer ramifications of nerves by staining agents in any situation, and second, the quite peculiar difficulty with which these methods are applied to an organ such as the testicle.

Hence the only observations on the terminations of the nerves within the seminiferous tubules of the testicle at present on record are those of Letzerich[1] (at least to my knowledge).

Inasmuch as the result of my own investigation leads me to differ very much from the views held by this observer, I will briefly mention the principal points of his experiments.

L. uses either fresh seminiferous tubules or such as have been in a solution of very dilute chromic acid ($\frac{1}{25}$ per cent.)

[1] *Virchow's Archive*, Bd. 42.

for the period of twenty-four hours; then he teases them out carefully with needles, and examines them with the microscope. Under favorable circumstances, he finds that the nerves approach the membrana propria, perforate the same, and finally terminate in granular masses or knobs between the latter membrane and the first layer of cells.

Although I have given his experiments a very careful consideration and a most extended repetition, using the testicles of various animals, and have availed myself of the rules laid down by him in preparing them for microscopic examination, I must say that circumstances never favored me in finding the structures pictured in the plates accompanying his article. In this I have been no less unfortunate than Von La Valette St. George,[1] who, likewise, was not able to satisfy himself as to the correctness of L.'s observations.

Methods.—The methods of investigation followed out in my researches require a brief notice. The testicles used were those of the dog, cat, calf, mouse, rat, rooster, and man. Both teasing and section cutting were practised; the former method, however, I found after a while quite superfluous, and finally confined myself to section cutting only. The tissues from which the material for study was collected were both fresh and hardened; the latter was done by alcohol, or chromic acid, or picric acid. The staining agents which I found of most advantage were chloride of gold, osmic acid and picro-carmine, and eosin with log-wood.

For the study of the nerve fibres outside of the seminiferous tubules, and their plexuses around the tubules, arising from the larger nerve-bundles which pass along the small arterioles, osmic acid and picro-carmine have in my hands given good results. I can also recommend eosin with log-wood, for the same purpose, as deserving a trial. The action of osmic acid brings out a sharply defined outline in the

[1] Stricker's "Hand-book of Histology."

anatomical structure of the non-medullated nerve fibres by surrounding each fibre with a dark border strongly refracting the light, the body of the nerve taking on a faintly pinkish hue. This dark border seems to point to the existence of a very fine and delicate membrane surrounding non-medullated nerve fibres. The nature of this membrane has points in common with the sheath of Swann, which surrounds medullated nerve fibres.

The chloride of gold method is still the only available one for bringing out the ultimate nerve terminations, the so-called axis-fibrillæ. The original unmodified method devised by Jul. Cohnheim, has been found to give excellent results. Some of the most beautiful and, at the same time, convincing specimens in my possession are sections of the testicles of a young rat, which were hardened in chromic acid and then stained with chloride of gold in strict accordance with the rules layed down by Cohnheim.

A circumstance in connection with the method perhaps worth mentioning, is that specimens prepared as described above will certainly show improvement during the first twelve months. Some which were mounted a year ago in glycerine and laid aside exhibited very fine markings of axis-fibrillæ, which they had not done formerly.

It was certainly a step in advance when Löwitt introduced his formic acid method, by which we are enabled to allow the reduction of gold to take place in a darkened bottle. This plan, in some cases, gives better and much more uniform results, and reduces the metallic precipitate so often found on the surface of sections to a minimum, but the destruction of the epithelia which is entailed is not in all cases desirable.

Ranvier's lemon-juice and formic acid process, as recommended by Stirling,[1] may also be tried with good success.

[1] "Handbook of Histology."

In hardening, staining, and preparing tissues generally, I have preferred to cut up the testicles into sections of from 2–3 millimetres in thickness, and, instead of the embedding methods, have found it much more convenient to use the freezing microtome for fine sections. In so doing, shrinkage, and the introduction of foreign material which must more or less alter either chemically or physically all organic matter, are avoided.

Of course, it often happens that when one prepares and stains thick sections, and afterward cuts them into thin microscopic ones, all specimens are not equally or thoroughly stained throughout, which is a disadvantage, but by a little practice the process can be regulated so as to obviate this difficulty to a great extent; in this way much larger and thinner sections can be obtained.

Being obliged to use high powers for the recognition of the ultimate termination of the axis-fibrillæ, the thinnest sections were chosen and mounted in glycerine to which one third of its volume of distilled water had been added.

Specimens stained with osmic acid and picro-carmine (more especially when the latter stain predominates) can be advantageously treated after the plan proposed by Prof. Neumann,[1] of Kœnigsberg, by temporarily mounting in glycerine mixed with muriatic acid in the proportion of 200 parts of the former to one of the latter, and carefully watching until the orange-red coloration has been reduced to the nucleus, then washing out thoroughly in distilled water. Great care, however, must be taken in this procedure in removing all the acid before permanently mounting in pure glycerine. One of the objections to picro-carmine has been that it does not bring out the nuclei as well as some other staining agents; the plan of this observer is well calculated, more particularly in pure picro-carmine specimens, to meet this objection and becomes, therefore, of value.

[1] *Waldeyer's Archiv.*, Bd. 18, 1880, p. 130.

The Spermatic Plexus of Nerves:—All anatomists agree that the nerves of the testicle are derived from the sympathetic. As long ago as 1834, Joseph Swan[1] gave a very good representation of the spermatic plexus of nerves. According to Robert B. Todd,[2] the nerves of the testicle are derived chiefly from the renal plexus, but partly also from the sup. mesenteric and aortic plexuses. These nerves then descend in company with the spermatic artery to the cord, where, being joined by branches from the hypogastric plexus which pass along the vas deferens, they form together the spermatic plexus. The branches of this plexus are intermingled with the vessels of the cord, and ultimately terminate within the substance of the testis. A few twigs may also be traced to the coverings of the gland.

Sappey[3] recognizes two sources of nerve supply, namely: one from the plexus accompanying the spermatic artery, which, he says, alone penetrates into the substance of the testis; and the other from the plexus surrounding the vas deferens which, according to his view, terminates in the epididymis.

In regard to the nerves running within the structure of the testis, I can corroborate the views above detailed: namely, that none but non-medullated nerve fibres are found, and I can add that their characteristic arrangement is in the shape of plexuses (see fig. 1). These when found in the neighborhood of the larger arterioles are, of course, large in proportion. As they pass on, always accompanying the blood-vessels, they, by division and frequent branching, become more numerous and very much smaller, until finally, after having reached the capillaries, they are extremely thin and transparent, and almost escape the observer's eye in the fresh and unstained specimen. In

[1] "A Demonstration of the Nerves of the Human Body," London, plate v.

[2] "Cyclop. of Anat and Phys.," vol. iv, pt. 2, p. 982.

[3] "Anatomie Descript.," tome 4, p. 614.

successful sections, however, they can still be seen as retaining the plexiform arrangement. When found in the proximity of a seminiferous tubule, they are generally situated between a capillary and the basement membrane. As they penetrate this membrane, the nerve fibres, still including several axis-cylinders, break up into their ultimate fibrillæ, at first pass along between the several layers of endothelia of which the basement membrane is composed,[1] and, after having emerged from its inner wall, they, as it were, line its interior with a plexus composed of the ultimate axis-fibrillæ, being only interrupted by variously shaped bodies, most of which present a pyramidal shape. This plexus, thus lining the inner surface of a seminiferous tubule, is best observed in the testicles of animals in which the membrana propria is very thin and composed of but one layer of endothelia, such as the mouse and rat. From this plexus, best seen in gold preparations, viz., longitudinal sections which have lost most of their epithelium, so as to expose the inner surface of the seminiferous tubule (see fig. 2), the axis-fibrillæ pass upward at acute angles with the surface in a direction toward the centre or lumen of the tubule, and between the epithelia superimposed upon the basement membrane or membrana propria; the fibrillæ anastomose in every direction, and hold, so to say, the epthelia in a mesh-work. This may be called the terminal or intratubular plexus, in contradistinction to the plexus surrounding the outer wall of a seminiferous tubule, which is called the extratubular plexus.

The cement substance between the epithelia is the place of the ultimate termination of the axis fibrillæ in the testicle. I have never seen a nerve fibre penetrate into the interior of an epithelium, althought it might seem so when one of them crosses an epithelium and is interrupted in its

[1] Berichte über die Verhandlungen der Kœniglich Sächsischen Gesellschaft der Wissenschaften zu Leipzig, 1873.

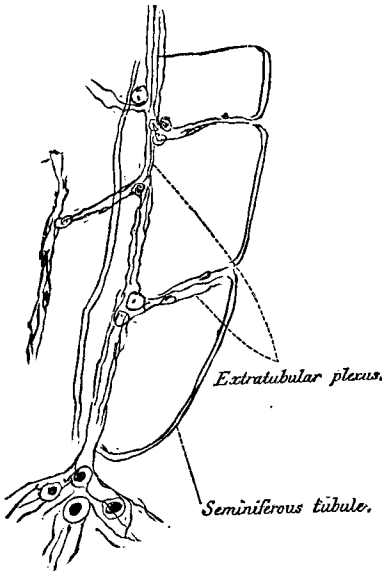

FIG. 1.—Testicle of dog, showing arrangement of extra-tubular plexus of nerve fibres. Gold preparation × 400.

FIG. 3.—Testicle of dog, showing terminal plexus running in cement substance between epithelia, in which traces of intra-epithelial reticulum may be seen. Gold preparation × 1000.

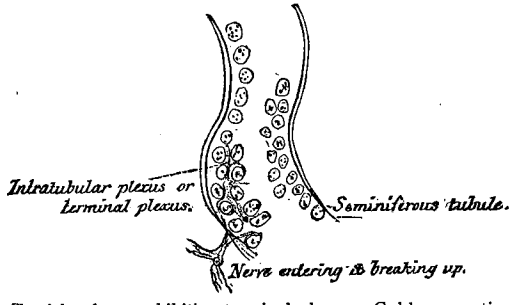

FIG. 2.—Testicle of rat, exhibiting terminal plexus. Gold preparation × 400.

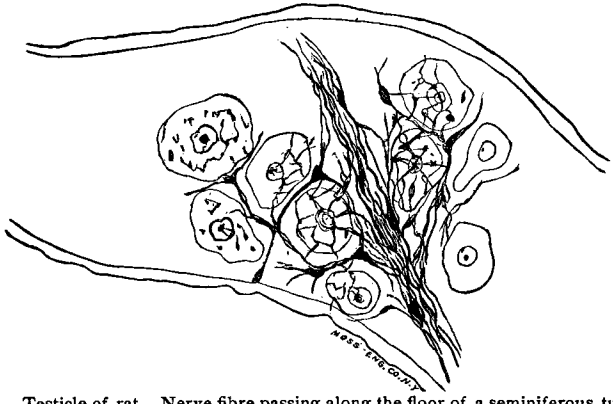

FIG. 4.—Testicle of rat. Nerve fibre passing along the floor of a seminiferous tubule and breaking up into the ultimate fibrillar plexus. Gold preparation × 1000.

course. The plexus, then, is the ultimate termination of the sympathetic in the testicle.

A satisfactory explanation for this termination of nerves in the testicle is offered by the discovery of Dr. Carl Heitzmann of the existence of the intra-epithelial network of living matter within all plastids including epithelia, a discovery which has been corroborated by careful observers, such as E. Klein and others, and has become generally recognized as a matter of no doubt. The only question at present agitating the minds of some investigators is that of the biological importance which this reticulum implies.

According to the researches of Dr. Carl Heitzmann, the reticulum within the epithelia, as well as the thorns or prickles traversing the cement substance between them, the two being in direct connection with one another, are the living or contractile matter proper. The ultimate axis-fibrillæ having been traced traversing the cement substance between the epithelia and being connected with the filaments crossing it, we are at once in a position to understand in what manner the function of the epithelia within the seminiferous tubules, viz., the production of spermatozoa, is directly controlled by the sympathetic nervous system.

U. S. Naval Hospital,
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